inject an aliquot of the supernatant. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water, pH 8.0.)

HPLC VARIABLES
Guard column: 10 μm RP-18
Column: 150 × 4.6 5 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 1.5:98.5 (Buffer was 10 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)
Flow rate: 1.5
Detector: F ex 340 em 455 following post-column reaction with derivatization reagent pumped at 0.9 mL/min. (Derivatization reagent was commercially available (Pierce) or prepared by adding 2.5 mL 2-mercaptoethanol and 2.5 mL Brij-35 to 850 mg o-phthalaldehyde in 10 mL MeOH, mix until decolorization is complete, add 1 L buffer, filter (0.45 μm), and refrigerate until used. Buffer was prepared by adjusting pH of 250 mM boric acid to 9.5 with 5 M KOH.)

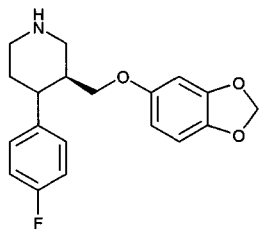
CHROMATOGRAM
Retention time: 19

OTHER SUBSTANCES
Extracted: neomycin
Simultaneous: dihydrostreptomycin, streptomycin

KEY WORDS
kidney; muscle; cow; pig; post-column reaction

REFERENCE
Shaikh,B.; Allen,E.H.; Gridley,J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36.

Paroxetine



Molecular formula: $C_{19}H_{20}FNO_3$
Molecular weight: 329.37
CAS Registry No.: 61869-08-7
Merck Index: 7175
Lednicer No.: 5 87

SAMPLE
Matrix: blood
Sample preparation: Condition a 50 mg Carboxymethyl Isolute SPE cartridge with 1 mL MeOH and 1 mL 25 mM pH 6.8 phosphate buffer, dry under vacuum. Add 500 μL plasma to the SPE cartridge, wash with two 1 mL portions of 25 mM pH 6.8 phosphate buffer, dry under vacuum, elute with 1 mL 1% ammonia in MeOH, evaporate to dryness under vacuum at 40°, reconstitute the residue in 100 μL MeOH, vortex, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS/CN
Mobile phase: MeOH:50 mM pH 4.8 potassium phosphate buffer 70:30
Flow rate: 1
Injection volume: 25
Detector: E, ESA, Model 5100 A, Model 5010 analytical cell +650 mV on channel 1, +950 mV on channel 2, Model 5020 guard cell +980 mV

CHROMATOGRAM
Retention time: 9.6
Internal standard: paroxetine

OTHER SUBSTANCES
Extracted: desipramine, venlafaxine

KEY WORDS
plasma; SPE; paroxetine is IS

REFERENCE
Clement,E.M.; Odontiadis,J.; Franklin,M. Simultaneous measurement of venlafaxine and its major metabolite, oxydesmethylvenlafaxine, in human plasma by high-performance liquid chromatography with coulometric detection and utilisation of solid-phase extraction, *J.Chromatogr.B*, **1998**, *705*, 303–308.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 250 μL buffer + 200 μL water + 100 μL 25 ng/mL maprotiline in water + 4 mL toluene, extract on a tumble mixer for 15 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 50 μL acetone, add 25 μL 100 mM sodium bicarbonate, add 10 μL 1 mg/mL dansyl chloride in acetone (prepare fresh daily), vortex for 15 s, heat at 55° for 1 min, centrifuge for 1 min, let stand at room temperature for 30 min, add 25 μL 25 mg/mL L-proline in water (prepare fresh daily), vortex briefly, centrifuge for 1 min, let stand at room temperature for 5 min, add 500 μL water, add 2 mL toluene, agitate on a tumble mixer for 10 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 55°, reconstitute the residue in 100 μL mobile phase, vortex for 30 s, inject an aliquot. (Buffer was 8.6 g $Na_2HPO_4.12H_2O$ in 100 mL water, adjusted to pH 12.0 with 4 M NaOH, made up to 200 mL with water.)

HPLC VARIABLES
Guard column: 30 mm long 5 μm Spherisorb ODS
Column: 200 × 4 5 μm Spherisorb ODS
Mobile phase: MeOH:50 mM pH 4.5 sodium acetate buffer 84:16 (At the end of the day wash column with 95:5.)
Flow rate: 1.5
Injection volume: 100
Detector: F ex 340 em 520

CHROMATOGRAM
Retention time: 5.8
Internal standard: maprotiline (8.2)
Limit of detection: 0.2 ng/mL
Limit of quantitation: 0.5-1 ng/mL

OTHER SUBSTANCES
Noninterfering: cimetidine, digoxin, methyldopa, phenobarbital, phenytoin, procyclidine, tranylcypromine

KEY WORDS
plasma; derivatization; pharmacokinetics

REFERENCE
Brett,M.A.; Dierdorf,H.-D.; Zussman,B.D.; Coates,P.E. Determination of paroxetine in human plasma, using high-performance liquid chromatography with fluorescence detection, *J.Chromatogr.*, **1987**, *419*, 438–444.

SAMPLE
Matrix: blood

Sample preparation: 1 mL Plasma + 75 μL 1.3 μg/mL norfemoxetine in water + 200 μL 1 M perchloric acid + 5 mL toluene, extract in a tumble mixer for 20 min, centrifuge at 1500 g for 10 min, let stand at -20° for 20 min. Remove the aqueous phase and add it to 750 μL 25 mM pH 12 phosphate buffer and 200 μL 1% lauryl sulfate, add 5 mL heptane:toluene 80:20, extract on a tumble mixer for 20 min, centrifuge at 2000 g for 10 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 220-250 μL MeOH:pH 4.5 acetate buffer 30:70, mix thoroughly, inject a 180-200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 RP-Select B
Mobile phase: MeCN:EtOH:buffer 21:14:65 (Buffer was 50 mM acetic acid adjusted to pH 4.5 with 1 M NaOH containing 2 g/L (?) tetrabutylammonium hydrogen sulfate.)
Flow rate: 1
Injection volume: 180-200
Detector: UV 295

CHROMATOGRAM
Retention time: 11.5
Internal standard: norfemoxetine (9.5)
Limit of quantitation: 6 ng/mL

KEY WORDS
plasma

REFERENCE
Knoeller,J.; Vogt-Schenkel,R.; Brett,M.A. A simple and robust HPLC method for the determination of paroxetine in human plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 635–638.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.275

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: dialysate
Sample preparation: Inject dialysate onto column A and column B in series, elute with mobile phase, monitor the effluent from column B, after 15.3 min remove column B from the circuit, continue to elute column A with mobile phase, monitor the effluent from column A. (Serotonin elutes from column A and column B. The more highly retained paroxetine is eluted from the shorter column A.)

HPLC VARIABLES
Column: A 50 × 2.0 μm Nucleosil C18; B 250 × 2.1 5 μm Supelcosil LC-18-DB (Supelco, USA)
Mobile phase: MeCN:buffer 33:67 (Buffer was 0.23 mM 1-octanesulfonic acid sodium salt in 65 mM acetic acid, adjusted to pH 2.8 with glacial acetic acid.)
Flow rate: 0.127 for 13.7 min then 0.4
Injection volume: 10
Detector: F ex 280 em 340

CHROMATOGRAM
Retention time: 18.5
Limit of detection: 300 fmol
Limit of quantitation: 4.2 pmol

OTHER SUBSTANCES
Extracted: serotonin

KEY WORDS
brain; column switching; rat

REFERENCE
Ramaiya,A.; Karnes,H.T. Simultaneous measurement of serotonin and paroxetine in rat brain dialysate by a single-pump column-switching technique, *J.Chromatogr.B*, **1997**, *691*, 119–129.

SAMPLE
Matrix: formulations
Sample preparation: Extract tablets with mobile phase so as to give a paroxetine concentration of 400 μ g/mL.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil C18
Mobile phase: MeCN:buffer 40:60 (Buffer was 10 mM 1-decanesulfonic acid sodium salt containing 10 mM NaH_2PO_4, pH 3.0.)
Detector: UV 235

CHROMATOGRAM
Limit of quantitation: 800 ng/mL

OTHER SUBSTANCES
Extracted: degradation products

KEY WORDS
tablets; stability-indicating

REFERENCE
Lambropoulos,J.; Spanos,G.A.; Lazaridis,N.V. Method development and validation for the HPLC assay in paroxetine 20 mg strength tablets (Abstract 3391), *Pharm.Res.*, **1997**, *14*, S591.

SAMPLE
Matrix: formulations
Sample preparation: Weight out powdered tablets equivalent to 20 mg paroxetine. Suspend the powder in three 5 mL portions of MeOH, stir for 30 min, filter, dry under a gentle stream of nitrogen. Reconstitute the residue in 20 mL 2-propanol. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Chiralpak AD
Mobile phase: n-Hexane:EtOH:diethylamine 94:6:0.5 (At the end of each day wash column with ca. 100 mL n-hexane:2-propanol 90:10.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 296

CHROMATOGRAM
Retention time: 39 (+), 45 (-)
Limit of detection: 2 ng
Limit of quantitation: 6 ng

KEY WORDS
chiral; tablets

REFERENCE
Ferretti,R.; Gallinella,B.; La Torre,F.; Turchetto,L. Validated chiral high-performance liquid chromatographic method for the determination of trans-(-)-paroxetine and its enantiomer in bulk and pharmaceutical formulations, *J.Chromatogr.B*, **1998**, *710*, 157–164.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

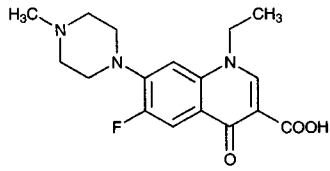
CHROMATOGRAM
Retention time: 11.06 (A), 5.77 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Pefloxacin



Molecular formula: $C_{17}H_{20}FN_3O_3$
Molecular weight: 333.36
CAS Registry No.: 70458-92-3, 70458-95-6 (mesylate)
Merck Index: 7197
Lednicer No.: 4 141

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma with 20 µL 20 µg/mL IS in MeOH. Vortex for 1 min with dichloromethane:diethyl ether 80:20, centrifuge at 1000 g for 10 min, separate the organic layer. Add 4 mL dichloromethane-diethyl ether 80:20, repeat the same extraction procedure twice, evaporate the organic phase to dryness under a stream of nitrogen, add 100 µL 10 mM NaOH to the residue, shake for 5 min, inject a 20 µL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 10 µm Vydac AXGU
Column: 250 × 4.6 5 µm Adsorbosphere SAX
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 10:90
Flow rate: 1.2
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 9.2
Internal standard: 2-[4-(2-furoyl)phenyl]propionic acid (3.9)
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: felbinac, fenbufen

KEY WORDS
plasma

REFERENCE
Carlucci,G.; Palumbo,G.; Mazzeo,P. Simple and rapid analysis of pefloxacin, fenbufen and felbinac in human plasma using high-performance liquid chromatography, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 1107–1115.

SAMPLE
Matrix: blood
Sample preparation: 200 µL Serum + 50 µL 400 µg/mL IS in water, vortex for 30 s. Add 500 µL MeCN, vortex for 1 min. Centrifuge at 6000 rpm for 10 min. Evaporate the supernatant to 200 µL at 40° under a stream of nitrogen, vortex for 30 s. Inject a 30-80 µL aliquot.

HPLC VARIABLES
Column: 100 × 8.0 4 µm Radial-pak Novapak C18
Mobile phase: MeCN:buffer 14:86 (Buffer was 2 g citric acid, 2 g sodium acetate, and 1 mL triethylamine in 1 L water.)
Flow rate: 2.5
Injection volume: 30-80
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 5.2
Internal standard: acebutolol (7.4)
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Extracted: norfloxacin
Simultaneous: ciprofloxacin, lomefloxacin, ofloxacin

KEY WORDS
serum; pharmacokinetics

REFERENCE
Abanmi,N.; Zaghloud,I.; El Sayed,N.; al-Khamis,K.I. Determination of pefloxacin and its main active metabolite in human serum by high-performance liquid chromatography, *Ther.Drug Monit.*, **1996**, *18*, 158–163.

SAMPLE
Matrix: blood
Sample preparation: Filter 1 mL plasma using a micropartition system (MPS-1, Amicon, MA) while centrifuging at 2000 g for 20 min at 10°, inject an aliquot of the ultrafiltrate.

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb ODS-2 endcapped
Mobile phase: MeCN:buffer 11:89 containing 5 mM tetrabutylammonium sulfate, adjusted to pH 2.5 with 1 M NaOH (Buffer was 100 mM citric acid containing 200 mM ammonium perchlorate.)
Column temperature: 37
Flow rate: 1.2
Detector: UV 272

CHROMATOGRAM
Retention time: 9.97
Internal standard: pipemic acid (4.14)

OTHER SUBSTANCES
Simultaneous: norfloxacin

KEY WORDS
plasma; ultrafiltrate

REFERENCE
Zlotos,G.; Bücker,A.; Kinzig-Schippers,M.; Sorgel,F.; Holzgrabe,U. Plasma protein binding of gyrase inhibitors, *J.Pharm.Sci.*, **1998**, *87*, 215–220.

SAMPLE
Matrix: blood
Sample preparation: 500 μL Serum + 250 μL 10% trichloroacetic acid, vortex for 10 s, centrifuge at >700 g for 10 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 150 × 3.9 Nova-Pak C18
Mobile phase: MeOH:18 mM KH_2PO_4 containing 0.13 mM heptanesulfonic acid:concentrated phosphoric acid 30:70:0.1
Injection volume: 20
Detector: F ex 277 em 475

CHROMATOGRAM
Retention time: 6.5

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum

REFERENCE
Griggs,D.J.; Wise,R. A simple isocratic high-pressure liquid chromatographic assay of quinolones in serum, *J.Antimicrob.Chemother.*, **1989**, *24*, 437–445.

SAMPLE
Matrix: blood
Sample preparation: Add two volumes of MeCN to plasma, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 5 μm Nucleosil C18
Mobile phase: MeOH:100 mM pH 4.9 phosphate buffer 50:50
Column temperature: 40
Flow rate: 1.2
Detector: F ex 275 em 415

CHROMATOGRAM
Limit of quantitation: 78 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Jaehde,U.; Sörgel,F.; Stephan,U.; Schunack,W. Effect of an antacid containing magnesium and aluminum on absorption, metabolism, and mechanism of renal elimination of pefloxacin in humans, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1129–1133.

SAMPLE
Matrix: blood, dialysate
Sample preparation: 100 μL Plasma or dialysate + 400 μL MeOH, vortex, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: strong cation exchange
Mobile phase: MeCN:100 mM pH 3 citrate buffer 20:80
Detector: F ex 278 em 440

CHROMATOGRAM
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Rose,T.F.; Bremner,D.A.; Collins,J.; Ellis-Pegler,R.; Isaacs,R.; Richardson,R.; Small,M. Plasma and dialysate levels of pefloxacin and its metabolites in CAPD patients with peritonitis, *J.Antimicrob.Chemother.*, **1990**, *25*, 657–664.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. Mix 500 μL plasma with 5 μg IS, add 4 mL dichloromethane and 100 μL pH 7.4 phosphate buffer, agitate for 10 min, centrifuge at 5300 g for 10 min. Collect 3.5 mL organic phase. Add 4 mL dichloromethane to the aqueous phase again, agitate, centri-

fuge. Combine the organic phases, evaporate at 60°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot. Tissue. Mix 500 μL epiploic-fat and 4 mL dichloromethane and keep at 4°, add 5 μg IS, mix by using an automatic grinder (Ultra Turrax, Ika-Werk, Stauffen, Germany). Collect the mixture, centrifuge at 5300 g for 10 min. Add 4 mL 100 mM NaOH to the dichloromethane, agitate for 10 min, centrifuge at 5300 g for 5 min. Eliminate the organic phase, adjust the aqueous phase to pH 7.4 with concentrated trichloroacetic acid, add 4 mL dichloromethane, agitate for 10 min and centrifuge at 5300 g for 5 min. Evaporate the organic phase at 60°. Reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 4 5 μm 100 RP-18 Lichrosphere
Column: 125 × 4 5 μm 100 RP-18 endcapped Lichrosphere
Mobile phase: MeCN:pH 4.8 citrate buffer 85:15
Flow rate: 1
Injection volume: 20
Detector: F ex 330 em 418

CHROMATOGRAM
Internal standard: 4844P (pefloxacin analog)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: norfloxacin

KEY WORDS
epiploic-fat; plasma; pharmacokinetics; fat

REFERENCE
Jacoberger,B.; Ubeaud,G.; Freys,G.; Pottecher,T.; Jung,L.; Koffel,J.C. Concentrations of pefloxacin in plasma and tissue after administration as surgical prophylaxis, *Antimicrob.Agents Chemother.*, **1998**, *42*, 425–427.

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Briefly vortex 500 μL plasma and 500 μL pH 7.4 phosphate buffer, add 5 mL dichloromethane:isopropanol 80:20, vortex for 30 s, shake gently for 15 min on an electric shaker, centrifuge at 1500 g for 10 min. Separate the lower layer using phase-separating filter paper (Whatman 1 PS), evaporate the organic phase under nitrogen at 25°, dissolve the residue in 100 μL mobile phase, inject a 20μL aliquot. Tissue. Pulverize prostatic tissue under liquid nitrogen, weigh a 200 mg aliquot, add 500 μL pH 7.4 phosphate buffer and 5 mL dichloromethane:isopropanol 80:20, vortex for 30 s, shake gently for 15 min on an electric shaker, centrifuge at 1500 g for 10 min. Separate the lower layer using phase-separating filter paper (Whatman 1 PS), evaporate the organic phase under nitrogen at 25°, dissolve the residue in 100 μL mobile phase, inject a 20 μL aliquot. (The pH 7.4 phosphate buffer was 28.2 g K_2HPO_4 and 5.17 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Nucleosil C18
Mobile phase: MeCN:pH 2.1 buffer 20.9:79.1 (Prepare the mobile phase by dissolving 18.1 g citric acid and 4.1 g ammonium perchlorate in about 300 mL distilled water, add 209 mL MeCN, dilute to 1 L with water, and add 3 mL tetrabutylammonium hydroxide. Filter through a 0.45 μm HV Millipore filter.)
Flow rate: 0.9
Injection volume: 20
Detector: UV 340

CHROMATOGRAM
Retention time: 6.8
Internal standard: pefloxacin

OTHER SUBSTANCES
Extracted: enoxacin, 4-oxo-enoxacin

Noninterfering: amikacin, ciprofloxacin, fosfomycin, ofloxacin, rifampicin, roxithromycin, tobramycin, vancomycin

KEY WORDS
plasma; prostatic tissue; prostate; pefloxacin is IS

REFERENCE
Hamel,B.; Audran,M.; Costa,P.; Bressolle,F. Reversed-phase high-performance liquid chromatographic determination of enoxacin and 4-oxo-enoxacin in human plasma and prostatic tissue. Application to a pharmacokinetic study, *J.Chromatogr.A*, **1998**, *812*, 369–379.

SAMPLE
Matrix: blood, tissue
Sample preparation: Tissue. Homogenize 100-250 mg tissue with 5 mL 500 mM pH 7.0 phosphate buffer, remove a 1 mL aliquot, add 100 µL 10 µg/mL IS, mix, add 10 mL chloroform: isopentanol 90:10, shake for 15 min, centrifuge , repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 µL 1% ammonia, inject a 25 µL aliquot. Plasma. 250-500 µL Plasma + 100 µL 10 µg/mL IS + 1 mL 500 mM pH 7.0 sodium phosphate buffer, mix, add 10 mL chloroform:isopentanol 90:10, shake for 15 min, centrifuge, repeat extraction. Combine the organic layers and evaporate them to dryness under a stream of air at 60°, reconstitute the residue in 100 µL 1% ammonia, inject a 25 µL aliquot.

HPLC VARIABLES
Column: 100 × 5 10 µm Nucleosil C18
Mobile phase: MeCN:water 15:85 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, and 1 mL/L triethylamine
Flow rate: 2
Injection volume: 25
Detector: F ex 330 em 440

CHROMATOGRAM
Retention time: 4.8
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (Roger Bellon Laboratories) (6.6)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
prostate

REFERENCE
Montay,G.; Tassel,J.P. Improved high-performance liquid chromatographic determination of pefloxacin and its metabolite norfloxacin in human plasma and tissue, *J.Chromatogr.*, **1985**, *339*, 214–218.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 250 µL Plasma + 250 µL 10 µg/mL IS in water + 750 µL MeOH, stir, centrifuge at 2000 rpm for 5 min, inject a 50 µL aliquot of the supernatant. Urine. 500 µL Urine + 3.5 mL 8 µg/mL IS in water, inject a 50 µL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 Nucleosil C8
Mobile phase: MeCN:water:triethylamine:formic acid 11:87.5:0.1:1 containing 0.2% sodium acetate and 0.1% citric acid
Flow rate: 1
Injection volume: 50
Detector: F ex 280 em 450

CHROMATOGRAM
Internal standard: 1-ethyl-6-chloro-1,4-dihydro-7-(4-methyl-1-piperazinyl)-4-oxo-3-quinolinecarboxylic acid (RP 41983)
Limit of quantitation: 500 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Humbert,G.; Brumpt,I.; Montay,G.; Le Liboux,A.; Frydman,A.; Borsa-Lebas,F.; Moore,N. Influence of rifampin on the pharmacokinetics of pefloxacin, *Clin.Pharmacol.Ther.*, **1991**, *50*, 682–687.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 278.3

CHROMATOGRAM
Retention time: 8.942

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Dissolve 40 mg freeze-dried nanoparticles in 25 mL acetone:MeOH 90:10 containing a few drops of 100 mM HCl, filter (0.45 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Partisil 10-ODS-1 C18
Mobile phase: MeCN:10 mM KH_2PO_4:triethylamine 14:86:0.2
Flow rate: 1.5
Injection volume: 20
Detector: UV 275

OTHER SUBSTANCES
Simultaneous: ofloxacin (UV 292)

KEY WORDS
nanoparticles

REFERENCE
Fresta,M.; Puglisi,G.; Giammona,G.; Cavallaro,G.; Micali,N.; Furneri,P.M. Pefloxacine mesilate and ofloxacin-loaded polyethylcyanoacrylate nanoparticles: Characterization of the colloidal drug carrier formulation, *J.Pharm.Sci.*, **1995**, *84*, 895–902.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) eye tissue with 3 mL 50 mM pH 5.8 sodium phosphate-citrate buffer and IS, centrifuge. Add the supernatant to 7 mL chloroform, agitate, centrifuge at 1000 g for 10 min. Remove the lower organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL mobile phase, inject a 5-20 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 3 μm Nucleosil C8
Mobile phase: MeCN:water 26:74 containing 2 g/L sodium acetate trihydrate, 2 g/L citric acid monohydrate, 4 mL/L triethylamine, and 2 mL/L formic acid, pH 4.8
Flow rate: 1
Injection volume: 5-20
Detector: UV 280

CHROMATOGRAM
Retention time: 1.89
Internal standard: 1-allyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-methyl-1-piperazinyl)quinoline-3-carboxylic acid (?) 4662 P (Roger-Bellon Laboratories) (2.95)
Limit of detection: 5 ng

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
rabbit; eye; pharmacokinetics

REFERENCE
Cochereau-Massin,I.; Bauchet,J.; Faurisson,F.; Vallois,J.M.; Lacombe,P.; Pocidalo,J.J. Ocular kinetics of pefloxacin after intramuscular administration in albino and pigmented rabbits, *Antimicrob.Agents Chemother.*, **1991**, *35*, 1112–1115.

SAMPLE
Matrix: urine
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeOH:MeCN:100 mM pH 5.75 phosphate buffer 24.1:2.6:73.3
Flow rate: 1
Detector: F ex 275 em 415

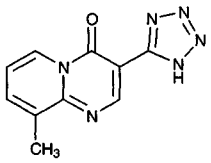
CHROMATOGRAM
Limit of quantitation: 780 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, norfloxacin

KEY WORDS
pharmacokinetics

REFERENCE
Jaehde,U.; Sörgel,F.; Stephan,U.; Schunack,W. Effect of an antacid containing magnesium and aluminum on absorption, metabolism, and mechanism of renal elimination of pefloxacin in humans, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1129–1133.

Pemirolast



Molecular formula: $C_{10}H_8N_6O$
Molecular weight: 228.21
CAS Registry No.: 69372-19-6, 100299-08-9 (potassium salt)
Merck Index: 7205
Lednicer No.: 5 150

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 25 μL 2.5 μg/mL IS in water + 1 mL MeOH, mix, centrifuge at 13000 g for 5 min, inject a 50 μL aliquot of the supernatant.

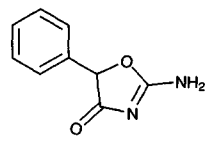
HPLC VARIABLES
Column: 100 × 9.4 5 μm ODS-3 RAC (Whatman)
Mobile phase: MeOH:water 45:55 containing 0.31% acetic acid
Flow rate: 1.5
Injection volume: 50
Detector: F ex 370 em 410 (360 nm cut-off filter)

CHROMATOGRAM
Retention time: 8.5
Internal standard: 7,9-dimethyl-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyrimidin-4-one (BL 5609) (Mead Johnson) (13.3)
Limit of detection: 0.4 ng/mL
Limit of quantitation: 4.3 ng/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Cheng,H.; Pittman,K.A.; Dandekar,K.A. Liquid chromatographic determination of 9-methyl-3-(1H-tetrazol-5-yl)-4H-pyrido[1,2-a]pyrimidin-4-one in human plasma with fluorescence detection, *J.Pharm.Sci.*, **1987**, *76*, 918–919.

Pemoline



Molecular formula: $C_9H_8N_2O_2$
Molecular weight: 176.17
CAS Registry No.: 2152-34-3
Merck Index: 7206

SAMPLE
Matrix: blood, tissue, urine

Sample preparation: Plasma. 100 μL Plasma + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 3 mL of the organic layer and add it to 1 mL 1 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot. Urine. 100 μL Urine + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 1 mL of the organic layer and add it to 1 mL 4 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, inject a 5 μL aliquot. Liver, kidney, lung, spleen, muscle. Homogenize on ice with 2 (liver, kidney, lung, spleen) or 3 (muscle) volumes ice-cold saline. 200 μL Homogenate + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 3 mL of the organic layer and add it to 1 mL 1 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, inject a 20 μL aliquot. Brain. Homogenize on ice with 2 volumes ice-cold saline. 200 μL Homogenate + 500 μL 1 M pH 10 carbonate buffer + 4 mL dichloromethane, shake for 10 min, centrifuge at 1680 g for 5 min. Remove 3 mL of the organic layer and add it to 1 mL 1 μg/mL 5-methyl-5-phenylhydantoin in dichloromethane, evaporate to dryness under reduced pressure at room temperature, reconstitute the residue in 50 μL mobile phase, centrifuge at 15300 g for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4 5 μm Kaseisorb LC C8-60-5 (Tokyo Kasei Kogo)
Mobile phase: MeCN:buffer 20:80 (Buffer was water adjusted to pH 5 with 15 mM phosphoric acid.)
Column temperature: 28
Flow rate: 0.7
Injection volume: 5-20
Detector: UV 215

CHROMATOGRAM

Retention time: 7
Internal standard: 5-methyl-5-phenylhydantoin (11)
Limit of detection: 5 ng (urine), 2 ng (tissue), 0.5 ng (plasma)

KEY WORDS

rat; plasma; brain; liver; kidney; lung; spleen; muscle; pharmacokinetics

REFERENCE

Aoyama,T.; Kotaki,H.; Saitoh,Y.; Nakagawa,F. Determination of pemoline in plasma, urine and tissues by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1988**, *430*, 351–360.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 1.50

OTHER SUBSTANCES

Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenyl-

ephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, phenazocine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

Interfering: benzphetamine, diethylpropion, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine

REFERENCE

Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recin-

namine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 4.91 (A), 3.84 (B)

OTHER SUBSTANCES

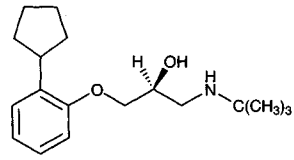
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, trifluopromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS

details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Penbutolol



Molecular formula: $C_{18}H_{29}NO_2$
Molecular weight: 291.43
CAS Registry No.: 38363-40-5, 38363-32-5 (sulfate)
Merck Index: 7209

SAMPLE
Matrix: blood
Sample preparation: 200 μL Plasma or serum + 50 μL 100 ng/mL protriptyline hydrochloride in 2 M pH 10.6 aqueous Tris buffer + 200 μL MTBE, vortex for 30 s, centrifuge at 9950 g for 2 min, inject a 100 μL aliquot of the organic layer.

HPLC VARIABLES
Column: 250 × 5 5 μm Spherisorb S5W
Mobile phase: Isooctane:MeOH:buffered MeOH:MTBE 55:15:10:20, apparent pH 5.7 (Buffered MeOH was 1 L 100 mM ammonium perchlorate in MeOH to which was added 10 mL 100 mM NaOH in MeOH, apparent pH 6.5.)
Flow rate: 2
Injection volume: 100
Detector: F ex 200 em no filter

CHROMATOGRAM
Retention time: 5.3
Internal standard: protriptyline (8)
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: N-acetylprocainamide, ajmaline, atenolol, betaxolol, chlorpromazine, desipramine, dipyridamole, doxazosin, flecainide, gallopamil, imipramine, labetalol, metoprolol, mianserin, nadolol, norverapamil, orphenadrine, oxprenolol, pindolol, prajmaline, prazosin, procainamide, propranolol, quinidine, quinine, terazosin, trazodone, triamterene, verapamil
Noninterfering: acebutolol, amiodarone, desethylamiodarone, disopyramide, lidocaine, lorcainide, methyldopa, nifedipine, propafenone, sotalol, timolol, tocainide
Interfering: mexiletine, pyrimethamine

KEY WORDS
plasma; serum; normal phase

REFERENCE
Bhamra,R.K.; Flanagan,R.J.; Holt,D.W. Measurement of penbutolol and 4-hydroxypenbutolol in plasma or serum by HPLC, *Biomed.Chromatogr.*, **1986**, *1*, 140–142.

SAMPLE
Matrix: blood
Sample preparation: 0.5-1 mL Plasma or 0.5 mL plasma water + 1 mL 1 M NaOH + 12 mL n-heptane:isoamyl alcohol 98.5:1.5, shake mechanically for 10 min, centrifuge at 1680 g for 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 40°, reconstitute the residue in 50 μL EtOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Hitachi Gel 3013 spherical styrene-divinylbenzene

Mobile phase: EtOH:buffer 65:35 (Buffer was 20 mM pH 2.0 perchloric acid/sodium perchlorate.)
Column temperature: 30
Flow rate: 0.2
Injection volume: 10
Detector: F ex 285 em 340

CHROMATOGRAM

Retention time: 18
Internal standard: penbutolol

OTHER SUBSTANCES

Extracted: propranolol
Simultaneous: quinidine, reserpine
Noninterfering: allopurinol, benzbromarone, diazepam, digoxin, diltiazem, dipyridamole, disopyramide, furosemide, isosorbide dinitrate, maprotiline, nifedipine, nitrazepam, trichlormethiazide, verapamil

KEY WORDS

penbutolol is IS; plasma; plasma water

REFERENCE

Yamamura,Y.; Uchino,K.; Kotaki,H.; Isozaki,S.; Saitoh,Y. Quantitative determination of propranolol in plasma and plasma water from normal subjects and patients with angina pectoris by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 311–319.

SAMPLE

Matrix: blood
Sample preparation: 100-500 μL Plasma + 5 ng bufarolol, mix, add 4 mL 500 mM pH 7.0 potassium phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 5 mL water, wash with 5 mL EtOH:water 30:70, elute with 5 mL EtOH:methylamine 99.9:0.1, evaporate to dryness, add 100 μL 2 mg/mL (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide in MeCN containing 0.1% quinuclidine, heat at 60° for 20 min, add 50 μL MeOH, evaporate to dryness under a stream of nitrogen, reconstitute with 1 mL in EtOH:water 90:10, add to an 18 × 6 column packed with 100 mg carboxymethyl Sephadex LH-200, wash with EtOH:water 90:10 at 0.2 mL/min, elute with 5 mL 100 mM methylamine in EtOH:water 90:10. Evaporate the eluate to dryness, reconstitute with 50-100 μL mobile phase, inject an aliquot. (Derivatization occurs on the alcohol. Preparation of (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide is as follows. Treat 1-bromo-2-naphthol with sodium hydride in DMF, add iodomethane, stir at room temperature overnight to obtain 1-bromo-2-methoxynaphthalene (mp 85-86°). Add a solution of 37.7 g 1-bromo-2-methylnaphthalene in 200 mL ether over 1 h to a sonicated mixture of 7 g magnesium turnings in 50 mL ether, the mixture should reflux rapidly (Caution! There may be in an induction period!), sonicate for 2 h after addition is complete, add 200 mL benzene (Caution! Benzene is a carcinogen!), add this mixture dropwise to a stirred mixture of 100 mmoles 1-bromo-2-methoxynaphthalene and 655 mg bis(triphenylphosphine)nickel(II) chloride ($NiCl_2(PPh_3)_2$) in 150 mL benzene at room temperature over 1 h, stir at room temperature overnight, reflux for 3 h, remove the ether by distillation through a short Vigreux column, remove the solvent by evaporation under reduced pressure, remove excess 1-bromo-2-methylnaphthalene by heating at 150°/0.1 mm Hg, cool, dissolve the residue in hexane, pass through silica gel, evaporate to dryness, recrystallize from hexane to obtain 1-methoxy-2'-methylbinaphthalene (mp 118-121°). Reflux 10 mmoles 1-methoxy-2'-methyl-binaphthalene, 1.96 g N-bromosuccinimide, and 100 mg benzoyl peroxide in 70 mL carbon tetrachloride for 3 h, filter, evaporate the filtrate to obtain crude 1-bromomethyl-2'-methoxy-binaphthalene. Dissolve the crude 1-bromomethyl-2'-methoxy-binaphthalene in 60 mL DMSO under nitrogen, slowly add a sodium ethoxide/nitropropane mixture, stir at room temperature for 3 h, stir at 60° for 3 h, pour into 300 mL ice-water, extract with dichloromethane, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to obtain crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde. (Prepare the sodium ethoxide/nitropropane mixture by dissolving 580 mg sodium in 35 mL EtOH, add 3.25 g 2-nitropropane.) Reflux the crude 2'-methoxy-1,1'-binaphthalene-2-carboxaldehyde in 60 mL acetone, add a solution of 2.36 g potassium permanganate in 60 mL hot water dropwise over 1 h, heat for an additional hour, pass sulfur dioxide through the solution until it becomes clear (sodium metabisulfite may work). Filter off the precipitate and dissolve it in 200 mL hot toluene, add a small amount of activated charcoal, filter while hot, concentrate to about a third of the volume, recrystallize

from EtOH:water 1:2 to obtain 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (mp 258.5-260°) (Bull. Chem. Soc. Japan 1986, 59, 2044). Reflux 9.15 g racemic 2'-methoxy-1,1'-binaphthalene-2-carboxylic acid in 55 mL freshly distilled thionyl chloride for 5 h, evaporate under reduced pressure, add a little benzene, evaporate under reduced pressure, repeat the benzene evaporation twice more to obtain 2'-methoxy-1,1'-binaphthalene-2-carbonyl chloride as a brown solid. Dissolve the acid chloride in 70 mL benzene, add dropwise to 12.8 g (-)-menthol in 100 mL benzene containing 1 g 4-dimethylaminopyridine and 5 mL pyridine, stir overnight at room temperature, heat at 70° for 3 h, cool, dilute with benzene, wash with 2 M HCl, wash with 1 M sodium carbonate, wash with water, dry over anhydrous magnesium sulfate in the presence of activated charcoal, evaporate to dryness, remove as much menthol as possible by sublimation under vacuum, chromatograph twice on a column of silica gel with toluene to obtain the (aS,R) menthol ester (mp 145-146° from hexane) and the (aR,R) menthol ester (mp 126-129° from hexane) as well as a mixture of diastereomers. Reflux the (aS,R) menthol ester with KOH in aqueous EtOH for 8-10 h to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carboxylic acid (Bull. Chem. Soc. Japan 1989, 62, 1528). Add 1.5 mL oxalyl chloride to a solution of (aS)-2'-methoxy-1,1'-binaphthalene-2- carboxylic acid in 10 mL anhydrous benzene, reflux for 10 h, evaporate to dryness under reduced pressure. Take up the residue in 10 mL anhydrous benzene, add 1 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 5 h, evaporate to dryness, recrystallize from hexane/acetone to obtain (aS)-2'-methoxy-1,1'-binaphthalene-2-carbonyl cyanide as orange-yellow needles (mp 143-146°).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Cosmosil 5SL (Nacalai Tesque, Kyoto)
Mobile phase: Hexane:ethyl acetate:triethylamine 83.3:16.7:0.005
Flow rate: 2
Detector: F ex 290 em 405

CHROMATOGRAM

Retention time: 10.5 (R), 13.5 (S)
Internal standard: bufarolol (8.5 (R), 12 (S))
Limit of detection: 30 pg

KEY WORDS

derivatization; plasma; chiral; normal phase; dog; SPE; pharmacokinetics

REFERENCE

Goto,J.; Shao,G.; Ito,M.; Kuriki,T.; Nambara,T. High-performance liquid chromatographic determination of penbutolol enantiomers in plasma with fluorescence detection, *Anal.Sci.*, **1991**, *7*, 723–726.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 270

CHROMATOGRAM

Retention time: 8.65
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Evaporate 0.5 mL 100 ng/mL propranolol in MeOH to dryness in a glass tube under a stream of nitrogen at 37°, add 1 mL plasma or urine, add 500 μL 1 M NaOH, add 8 mL freshly distilled diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 15 min. Remove 6.5 mL of the organic layer and pass it through a 20 × 4 column filled with glass wool, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot. For conjugated compounds proceed as follows. Evaporate 0.5 mL 100 ng/mL propranolol in MeOH to dryness in a glass tube under a stream of nitrogen at 37°, add 1 mL plasma or urine, add 1 mL 100 mM pH 5 acetate buffer, add 100 μL solution containing 10000 U/mL β-glucuronidase and 0.6 U/mL sulfatase (Sigma), heat at 37° for 48 h, cool to room temperature, add 500 μL 1 M NaOH, add 8 mL freshly distilled diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 15 min. Remove 6.5 mL of the organic layer and add it to 6 mL 100 mM HCl, shake at 60 rpm on a rotating shaker for 15 min, centrifuge at 1200 g for 10 min. Remove 5.5 mL of the aqueous layer and add it to 700 μL 1 M NaOH, add 6 mL diethyl ether, shake on a rotating shaker at 60 rpm for 15 min, centrifuge at 1200 g for 10 min, remove 5 mL of the organic layer, evaporate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 10 μm LiChrosorb RP-8

Mobile phase: MeCN:buffer 48:52 containing 1 g/L sodium heptanesulfonate (Buffer was 100 mM citric acid-sodium citrate buffer adjusted to pH 2.85 with 1 M HCl.)
Column temperature: 28
Flow rate: 1.7
Injection volume: 20
Detector: F ex 278 em 310

CHROMATOGRAM
Retention time: 7.75
Internal standard: propranolol (F ex 290 em 330) (4)
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 4-hydroxypenbutolol (F ex 290 em 330)

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Bernard,N.; Cuisinaud,G.; Sassard,J. Determination of penbutolol and its hydroxylated metabolite in biological fluids by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1982**, *228*, 355–361.

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Plasma + 100 µL 400 ng/mL propranolol in water + 20 µL 25 mg/mL ascorbic acid in water (prepare fresh daily) + 500 µL buffer, vortex, add 6 mL hexane:n-butanol 96:4, shake vigorously for 2 min, centrifuge briefly. Remove 5 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL MeOH:21 mM pH 5.5 ammonium acetate buffer 50:50, vortex, inject a 50 µL aliquot. To deconjugate samples proceed as follows. 300 µL Plasma or urine + 50 µL 200 mg/mL sodium bisulfite in water (prepare fresh daily) + 600 µL glucuronidase solution, vortex, flush tube with nitrogen, heat at 45 for 2 h, add 100 µL 400 ng/mL propranolol in water, add 20 µL 25 mg/mL ascorbic acid in water (prepare fresh daily), add 500 µL buffer, vortex, add 6 mL hexane:n-butanol 96:4, shake vigorously for 2 min, centrifuge briefly. Remove 5 mL of the organic layer and wash it with 2 mL buffer:25 mg/mL ascorbic acid 100:1. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 37°, reconstitute the residue in 200 µL MeOH:21 mM pH 5.5 ammonium acetate buffer 50:50, vortex, inject a 50 µL aliquot. (Buffer was prepared by mixing saturated sodium carbonate and saturated sodium bicarbonate to pH 9.4. Glucuronidase solution was 50000 U type G-0258 (abalone entrails) and 40000 U type L-II (limpet) glucuronidases (Sigma) in 10 mL 50 mM pH 5 acetate buffer.)

HPLC VARIABLES
Column: 250 × 4.6 Zorbax CN
Mobile phase: MeOH:THF:buffer 60:6:34 (Buffer was 0.8 g ammonium acetate in 340 mL water, pH adjusted to 5.5 with acetic acid (if necessary).)
Column temperature: 35
Flow rate: 1.5
Injection volume: 50
Detector: F ex 275 (slit width 6 nm) em 324 (slit width 10 nm)

CHROMATOGRAM
Retention time: 10
Internal standard: propranolol (8)
Limit of detection: 20 ng/mL (urine), 6 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: carazolol, metoprolol, physostigmine
Noninterfering: acetaminophen, aspirin, atenolol, bromocriptine, chloroquine, doxorubicin, hydrochlorothiazide, indomethacin, 17-methyltestosterone, nadolol, nandrolone, practolol, quinidine, salicyluric acid, sulfadiazine, sulfamerazine, sulfamethazine, timolol, triamterene, vinzolidine, warfarin

Interfering: pergolide

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Miner,D.J.; Binkley,D.A.; Bechtol,L.D. Liquid-chromatographic determination of penbutolol and its principal metabolites in plasma and urine, *Clin.Chem.*, **1984**, *30*, 717–723.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85: 15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 15.928

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 1.9

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan,

benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve 100 ng penbutolol and 200 μg (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide in 200 μL 0.01% quinuclidine in MeCN, heat at 60° for 10 min, inject an aliquot. (Synthesis of (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide is as follows. Reflux 210 g 1-bromo-2-methylnaphthalene, 160 g N-bromosuccinimide, 1 g benzoyl peroxide, and 250 mL carbon tetrachloride for 2.5 h, add 250 mL carbon tetrachloride, filter while warm, wash the residue several times with solvent. Concentrate and cool the filtrate to give 1-bromo-2-bromomethylnaphthalene (mp 230-240°) (J. Org. Chem. 1949, 14, 375). Dissolve 90 g 1-bromo-2-bromomethylnaphthalene in 400 mL chloroform, reflux, add 46.5 g powdered hexamine in portions, remove the hexaminium salt by filtration. Reflux this salt in 650 mL 50% acetic acid for 1 h, add 105 mL concentrated HCl, reflux for 5 min, cool, obtain 1-bromo-2-naphthaldehyde (mp 119-120°) by filtration. Heat 11 g 1-bromo-2-naphthaldehyde in 275 mL acetone at 60-68°, add a hot solution of 14 g potassium permanganate in 330 mL water over 30 min, heat for another 30 min, pass in sulfur dioxide (sodium metabisulfite ?) until the solution is clear, pour into water to give 1-bromo-2-naphthoic acid, purify by forming the ammonium salt and reprecipitating. Reflux 1-bromo-2-naphthoic acid in MeOH in the presence of sulfuric acid to give methyl 1-bromo-2-naphthoate. Heat methyl-1-bromo-2-naphthoate with copper bronze at 270-280° for 20 min, while still hot extract with toluene, cool to obtain dimethyl 1,1'-binaphthalene-2,2'- dicarboxylate, obtain more crystals by evaporating some of the solvent, recrystallize from EtOH to give dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate (mp 158°) (J. Chem. Soc. 1955, 1242). Add 8 g lithium tri-tert-butoxyaluminohydride in portions to 2.8 g dimethyl 1,1'-binaphthalene-2,2'-dicarboxylate in 150 mL anhydrous benzene:ether 50:50 (Caution! Benzene is a carcinogen!), heat at 80° for 2 h, acidify with 5% HCl. Remove the organic layer and dry it over anhydrous sodium sulfate, evaporate to dryness, chromatograph on 50 g silica gel with

hexane:ethyl acetate 80:20, recrystallize the product from hexane/acetone to give methyl 2-hydroxymethyl-1,1'-binaphthalene-2'-dicarboxylate (mp 117.5-118.5°). Add 5 mL 30% hydrogen bromide in acetic acid to 2 g methyl 2-hydroxymethyl-1,1'- binaphthalene-2'-dicarboxylate in 10 mL acetic acid, stir at 50° for 10 min, pour into ice-water, filter, chromatograph the solid on 40 g silica gel with hexane:ethyl acetate 30:1 to give methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate as pale yellow needles (mp 137-138°). Add 400 mg sodium borohydride to 1.9 g methyl 2-bromomethyl-1,1'-binaphthalene-2'-dicarboxylate in 10 mL DMSO, stir at 60° for 15 min, pour into ice-water, acidify with concentrated HCl, chromatograph the crude product on 40 g silica gel with hexane:ethyl acetate 10:1, recrystallize from MeOH to give methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate as colorless needles mp 97-98°. Add 30 mL 10% KOH to 1.2 g methyl 2-methyl-1,1'-binaphthalene-2'-carboxylate in 50 mL MeOH, reflux for 3 h, pour into ice-water, filter, recrystallize from hexane/ethyl acetate to give 2-methyl-1,1'-binaphthalene-2'-carboxylic acid as colorless needles (mp 232-233°). Add 4.1 g (-)-brucine in 20 mL EtOH to 3.3 g 2-methyl-1,1'-binaphthalene-2'-carboxylic acid dissolved in 60 mL EtOH, allow to stand overnight, filter, recrystallize the precipitate several times from EtOH. Add 5% HCl to the salt and extract with ethyl acetate, wash the organic layer with water, dry over anhydrous sodium sulfate, evaporate to dryness, recrystallize from hexane/acetone to give (-)-2-methyl-1,1'-binaphthalene-2'-carboxylic acid as colorless needles (mp 229-229.5°; $[\alpha]_D^{20}$ -41.3° (c = 0.58 in chloroform). Add 3 mL oxalyl chloride to 500 mg (-)-2-methyl-1,1'-binaphthalene-2'-carboxylic acid in 30 mL anhydrous dichloromethane, stir at room temperature for 2 h, evaporate to give an oily residue, take up in 10 mL dichloromethane, add 2 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, chromatograph on 5 g silica gel with hexane to give (-)-2-methyl-1,1'-binaphthalene-2'-carbonyl cyanide as a yellow oil ($[\alpha]_D^{20}$ -42.8° (c = 1.05 in chloroform).)

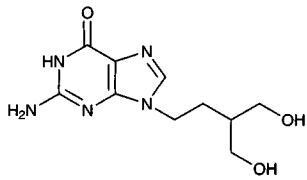
HPLC VARIABLES
Column: 150 × 4.6 5 μm spherical silica (Waters)
Mobile phase: Hexane:chloroform:MeOH 100:5:0.3
Detector: F ex 342 em 420

CHROMATOGRAM
Retention time: 11 (+), 12 (-)
Limit of detection: 200 pg

KEY WORDS
derivatization; chiral; normal phase

REFERENCE
Goto,J.; Goto,N.; Shao,G.; Ito,M.; Hongo,A.; Nakamura,S.; Nambara,T. Fluorescence chiral derivatization reagents for high performance liquid chromatographic resolution of enantiomeric hydroxyl compounds, *Anal.Sci.*, **1990**, *6*, 261–264.

Penciclovir



Molecular formula: $C_{10}H_{15}N_5O_3$
Molecular weight: 253.26
CAS Registry No.: 39809-25-1
Merck Index: 7210

SAMPLE
Matrix: reaction mixtures
Sample preparation: Filter (0.45 μm) a reaction mixture containing activated sludge, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-AQ C18 (YMC)
Mobile phase: MeOH:23 mM pH 7.0 potassium phosphate buffer 5:95
Column temperature: 30
Flow rate: 1

Injection volume: 20
Detector: UV 260

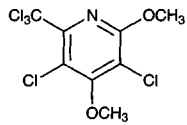
CHROMATOGRAM
Retention time: 17
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hsu,L.C.; Constable,D.J.C.; Orvos,D.R.; Hannah,R.E. Comparison of high-performance liquid chromatography and capillary zone electrophoresis in penciclovir biodegradation kinetic studies, *J.Chromatogr.B*, **1995**, *669*, 85–92.

Penclomedine



Molecular formula: $C_8H_6Cl_5NO_2$
Molecular weight: 325.41
CAS Registry No.: 108030-77-9

SAMPLE
Matrix: bile, blood, tissue
Sample preparation: Plasma, red cells. Add 1 mL plasma or red cells to 2.8 mL ethyl acetate and 200 μL 700 mM pH 2.7 ammonium phosphate buffer, homogenize, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen, add 50 μL MeCN, inject an aliquot. Liver slices. Add 1 mL incubation to 500 μL Krebs-Henseleit buffer containing 2.25% bovine serum albumin, homogenize, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen, add 50 μL MeCN, inject an aliquot. Bile. Dilute bile with an equal volume mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Adsorbosphere HS C18 (Alltech)
Mobile phase: Gradient. A was MeCN. B was 10 mM pH 2.7 ammonium phosphate buffer. A:B from 0:100 to 100:0 in 25 min, maintain at 100:0 for 15 min.
Flow rate: 1
Detector: UV 240; Radioactivity, Radiomatic Flo-One/Beta A140, with 500 μL cell, using Flo-Scint VI scintillant at ratio 2:1

CHROMATOGRAM
Retention time: 32

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
human; liver; mouse; ^{14}C labeled; plasma; red cells; erythrocytes

REFERENCE
Hartman,N.R.; Leo,K.U.; Brewer,T.G.; Strong,J.M. The in vitro metabolism of penclomedine in mouse, rat, and human systems, *Drug Metab.Dispos.*, **1998**, *26*, 513–519.

SAMPLE
Matrix: microsomal incubations

Sample preparation: Microsomes. Add 1 mL microsomal incubation to 2.8 mL ethyl acetate and 200 μL 700 mM pH 2.7 ammonium phosphate buffer, vortex, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen, add 50 μL MeCN to the residue, inject an aliquot. Liver slices. Mix 1 mL microsomal incubation with 500 μL Krebs-Henseleit buffer containing 2.25% bovine serum albumin, homogenize, centrifuge. Draw off the organic layer, add 50 μL dimethyl sulfoxide, evaporate to approximately 100 μL under nitrogen. Reconstitute the residue with 50 μL MeCN, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Adsorbosphere HS C18 (Alltech)
Mobile phase: Gradient. A was MeCN. B was 10 mM pH 2.7 ammonium phosphate buffer. A:B from 0:100 to 100:0 over 25 min, maintain at 100:0.
Flow rate: 1
Detector: UV 240; Radioactivity, Radiomatic Flo-One/Beta A140, with 500 μL cell, using Flo-Scint VI scintillant at ratio 2:1

CHROMATOGRAM

Retention time: 32

OTHER SUBSTANCES

Extracted: metabolites

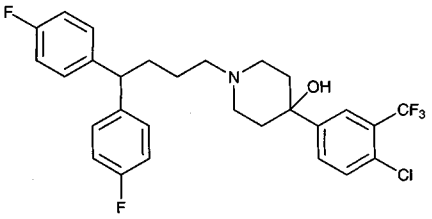
KEY WORDS

human; liver; mouse; ^{14}C labeled

REFERENCE

Hartman,N.R.; Leo,K.U.; Brewer,T.G.; Strong,J.M. The in vitro metabolism of penclomedine in mouse, rat, and human systems, *Drug Metab.Dispos.*, **1998**, *26*, 513–519.

Penfluridol



Molecular formula: $C_{28}H_{27}ClF_5NO$
Molecular weight: 523.97
CAS Registry No.: 26864-56-2
Merck Index: 7213
Lednicer No.: 2 334

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 266

CHROMATOGRAM

Retention time: 18.25

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

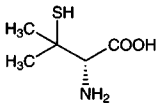
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.183

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Penicillamine



Molecular formula: $C_5H_{11}NO_2S$
Molecular weight: 149.21
CAS Registry No.: 52-67-5
Merck Index: 7214

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 150 μL 25% trichloroacetic acid, vortex, cool on ice for 10 min, centrifuge at 6500 g for 2 min. Remove a 500 μL aliquot of the supernatant and add it to 200 μL 1% NaOH in water, add 250 μL buffer, add 1 mL 1 mM N-[p-(2-benzoxazolyl)phenyl] maleimide (Eastman) in EtOH, heat at 37° overnight, inject a 50 μL aliquot. (Buffer was 500 mM sodium citrate adjusted to pH 5.0 with perchloric acid.)

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:100 μM sodium acetate 48:52
Flow rate: 2
Injection volume: 50
Detector: F ex 319 em 360 (cutoff filter)

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 250 nM
Limit of quantitation: 1 μM

KEY WORDS
derivatization; plasma

REFERENCE
Miners,J.O.; Fearnley,I.; Smith,K.J.; Birkett,D.J.; Brooks,P.M.; Whitehouse,M.W. Analysis of D-penicillamine in plasma by fluorescence derivatisation with N-[*p*-(2-benzoxazolyl)-phenyl] maleimide and high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *275*, 89–96.

SAMPLE
Matrix: blood
Sample preparation: Mix 1 mL plasma and 400 μL 18% (w/v) trichloroacetic acid in a 100 × 15 polypropylene tube, let stand at 0° for 5 min, centrifuge at 4° at 1700 g for 10 min, remove the supernatant as completely as possible. Suspend the precipitate in 1 mL 5% (w/v) trichloroacetic acid by stirring magnetically, centrifuge at room temperature at 2000 g for 5 min, discard the supernatant, repeat this washing step. Air-dry the precipitate then blanket it with nitrogen, add 2 mL 200 mM pH 8.0 Tris buffer, pass nitrogen over the mixture for 1 h, add 100 μL 250 mM EDTA, add 50 μL octanol, add 100 mg solid sodium borohydride, remove the